US009057720B2

(12) **United States Patent**
Verma

(10) **Patent No.: US 9,057,720 B2**
(45) **Date of Patent: Jun. 16, 2015**

(54) **REDUCING/OXIDIZING ACTIVITY OF MATERNAL URINE AS INDICATOR OF FETAL GENDER RELATED CHARACTERISTICS**

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(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 951 days.

(21) Appl. No.: **12/401,723**

(22) Filed: **Mar. 11, 2009**

(65) **Prior Publication Data**

US 2009/0233321 A1 Sep. 17, 2009

Related U.S. Application Data

(60) Provisional application No. 61/069,008, filed on Mar. 11, 2008.

(51) **Int. Cl.**

A61K 35/22	(2006.01)
C12Q 1/04	(2006.01)
G01N 33/50	(2006.01)
G01N 33/52	(2006.01)

(52) **U.S. Cl.**

CPC ***G01N 33/50*** (2013.01); ***G01N 33/52*** (2013.01)

(58) **Field of Classification Search**

None

See application file for complete search history.

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(57) **ABSTRACT**

The present invention provides a method for determining the gender of an unborn child by assaying the overall reducing/oxidizing or redox activity of the maternal urine or other body fluid. The method can be used to determine fetal gender at any time point during the entire pregnancy, the earliest being the first day of a missed menstruation. The body fluid may be processed before assaying. Processing may involve aging the body fluid, or purification of various fractions. The methods of the present invention also provide for a means for pre-conception baby gender planning by assaying the overall redox activity of a non-pregnant female's urine or other body fluid. The overall redox activity of a urine sample correlates with the gender specific compatibility of the ovum being released during a particular menstrual cycle. Therefore, assaying the overall redox activity of a non-pregnant female's urine will help a couple conceive a baby having a desired gender.

18 Claims, No Drawings

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REDUCING/OXIDIZING ACTIVITY OF MATERNAL URINE AS INDICATOR OF FETAL GENDER RELATED CHARACTERISTICS

CROSS REFERENCE TO RELATED APPLICATION

This application claims the benefit under 35 U.S.C. §119(e) of the U.S. Provisional Application No. 61/069,008, filed on Mar. 11, 2008.

FIELD OF THE INVENTION

The present invention relates to a method for determining the gender of an unborn child by evaluating the overall reducing/oxidizing activity of the maternal urine. The present invention also relates to a method for pre-conception baby gender planning by evaluating the overall reducing/oxidizing activity of a non-pregnant female's urine prior to conception.

BACKGROUND OF THE INVENTION

There is a great interest in accurately determining the gender of an unborn child as early as possible. Available techniques are typically performed at late stages of a pregnancy. Ultrasound can be used to determine fetal gender after the 18th week of pregnancy. Polymerase chain reaction (PCR) amplification of Y-specific DNA sequences requires a sample of maternal blood, as well as expensive equipment. PCR can be carried out as early as the 6th week of gestation with an accuracy of only around 80%. Lagona et al. *Multiple testing in fetal gender determination from maternal blood by polymerase chain reaction. Hum. Genet.* 102, 6:1 (1998). Amniocentesis is performed after the 18th week of pregnancy; however, it carries a risk of miscarriage due to its invasiveness. Chorionic Villi Sampling (CVS), conducted between the 10th and 13th week of pregnancy, can also provide accurate information, but again, the procedure is invasive and requires costly equipment.

Alternatively, it is possible to determine the gender of an unborn child by assaying sex hormones. Sex hormones are steroids that play important roles in both normal growth and development. In addition, sex hormones influence the development of sex organs and maintenance of secondary sex characteristics in mammals. Testosterone, the principal male sex hormone, is primarily secreted by the testes of males and, to a much less extent, the ovaries of females. On average, an adult man produces about forty to sixty times more testosterone than an adult woman. Similarly, estrogen functions as the primary female sex hormone and is usually present at significantly higher levels in women than men. The urine from a mother carrying a male fetus is richer in testosterone when compared with the urine from a mother carrying a female fetus. This difference is probably due to the contribution of sex hormones secreted by fetal testes. Jost A. *A new Look at the Mechanisms Controlling Sex Differentiation in Mammals. John Hopkins Med. J.* 130: 38 (1972). Approaches based on a pregnant woman's individual sex hormone levels have been explored to determine fetal gender; however, thus far, they have not resulted in the development of any statistically reliable methods. For example, in one study, antibodies against testosterone were used in a radio-immunoassay to determine the testosterone levels in pregnant mothers' urine. The accuracy of this study with respect to determining fetal gender was low. Loewit et al. *Determination of fetal sex from maternal testosterone excretion in early pregnancy. Dtsch. Med. Wschr.* 99: 1656 (1974).

Urine from pregnant women has been used in various attempts to develop simple and non-invasive tests to determine fetal gender. Most of these procedures exploit simple characteristics of maternal urine such as its pH or the ability to form complexes with aluminum compounds. Consequently, these tests are usually associated with comparatively poor reliability. For example, U.S. Pat. No. 6,420,182 discloses a method for fetal gender detection by assaying the pH of maternal urine after the 12th to 14th week of pregnancy. The test is based on the hypothesis that women bearing female fetuses have acidic urine, but the accuracy of this method is only about 65%. In another assay, a colorimetric test on urine from pregnant women may be performed after the 20th week of pregnancy. U.S. Pat. No. 4,840,914. The accuracy of this test in determining fetal gender was similarly low, only about 60%.

The applicant has noted that an evaluation of the reducing/oxidizing activity of a urine sample from a pregnant female provides a better method in determining fetal gender. Depending upon the processing and assay methods, the hormones which can contribute to the reducing activity of a urine sample are progesterone, testosterone and human chorionic gonadotropin (hCG), whereas estrogens can contribute to the oxidizing activity of the urine. The primary hormones directly linked to fetal gender are sex hormones, namely, testosterone and estrogens. The overall reducing/oxidizing activity of the urine sample from a pregnant mother carrying a male fetus is different from the overall reducing/oxidizing activity of the urine sample from a pregnant mother carrying a female fetus allowing for determination of fetal gender.

There is a continuing need to develop a simple, non-invasive and reliable method for determining the gender of an unborn child as early as possible after conception. There is also a need for pre-conception baby gender planning using a simple, non-invasive and reliable technique. Because human and other mammals have similar reproductive biology, these methods may also be of great commercial value to animal breeders.

SUMMARY OF THE INVENTION

It is an object of the present invention to provide a method for determining the gender of an unborn child comprising the steps of: (a) contacting a body fluid from a pregnant female with at least one redox indicator; (b) measuring redox activity of the body fluid; and, (c) comparing the redox activity of the body fluid with at least one standard to determine the gender of the unborn child. The body fluid may be selected from the group consisting of urine, tears, saliva, sweat, blood, plasma, serum, cerebrospinal fluid and amniotic fluid. The body fluid may be urine. The body fluid may be obtained during a period ranging from about the first day of a missed menstruation to about 40 weeks of pregnancy. The body fluid may also be obtained during a period ranging from about 5 weeks to about 15 weeks of pregnancy.

The redox indicator may comprise a heteropoly acid or its salts, such as phospho-24-tungstic acid. The redox indicator may be a chromogenic chemical. The chromogenic chemical may comprise an oxidation-reduction sensitive metallic ion. The metallic ion may be selected from the group consisting of copper, iron, chromium, tungsten and molybdenum ions in an oxidized state. The chromogenic chemical may be selected from the group consisting of ferric tripyridyltriazine (Fe(III)-TPTZ) complex and potassium ferricyanide. The redox activ-

ity may be measured using a free radical, such as hydroxyl radical. Sodium salicylate may be used as the redox indicator. The redox activity may also be measured using an electrochemical sensor. The redox activity may be measured on a solid substrate which comprises a redox indicator, such as a chromogenic chemical. The chromogenic chemical may be impregnated on a strip.

The body fluid may be processed prior to step (a). The body fluid may be aged for at least about 1 week at room temperature ranging from about 20° C. to about 30° C. prior to step (a). The body fluid may be aged at room temperature for a period of time ranging from about 1 week to 52 weeks prior to step (a). The processing of the body fluid may comprise chemical, biochemical, physical or biological means, such as enzymatic treatment, extraction and purification. The processing may comprise using adsorbants selected from the group consisting of talc, silica-based particles such as silica gel, alumina, florisil, charcoal, kaolin, concanavaline A and its conjugates, calcium phosphate, calcium hydroxide, calcium chloride, Cetyltrimethyl ammonium bromide, lectin, protein or glycoprotein hydrolyzing enzymes, glassfiber filter, ion exchange resins, affinity ligands, organic solvents, solid phase extractants, size exclusion sieves, and reverse phase chromatographic materials. The processing may also comprise using precipitants, such as heavy metals selected from the group consisting of barium, lead, molybdenum and tungsten. The precipitants may also be selected from the group consisting of barium chloride, barium hydroxide, zinc chloride, mercuric chloride, lead acetate, ammonium sulphate, dextran, acetonitrile, chloroform, sodium hydroxide, trichloroacetic acid, potassium iodate and their mixtures.

The present invention also provides a kit comprising, (a) a urine collecting vial; (b) a solid substrate comprising a redox indicator, wherein the redox indicator comprises at least one chromogenic chemical; (c) printed material instructing a woman to collect urine and contact the urine with the solid substrate; and (d) printed material instructing a woman to compare redox activity of the urine sample with at least one standard to determine the gender of the unborn child based on change in color in the chromogenic chemical. The kit may further comprise chemicals to process urine. The printed material may further instruct a woman to process the urine prior to contact the urine with the solid substrate.

The present invention further provides a method for preconception baby gender planning comprising the steps of: (a) contacting a body fluid from a non-pregnant female with at least one redox indicator; (b) measuring redox activity of the body fluid; and (c) comparing the redox activity of the body fluid with at least one standard to determine gender specific compatibility of the ovum released in a menstrual cycle. The body fluid may be urine. The body fluid may be obtained near ovulation or middle of the menstrual cycle. The body fluid may be processed prior to step (a).

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides a method for determining the gender of an unborn child by assaying the overall reducing/oxidizing or redox activity of the maternal urine.

The overall redox activity may be measured in other body fluids such as tears and cerebrospinal fluid. The method can be used to determine fetal gender at any time point during the entire pregnancy, i.e., from the first day of a missed menstruation to about 40 weeks of pregnancy. In order to improve the accuracy of results, the body fluid may be processed before assaying. Processing may involve aging the body fluid, or purification of various fractions. For example, processing can comprise aging the urine by storing it at ambient temperature (e.g., approximately, 20-30° C.) for a defined period of time, e.g., 1-4 weeks, treating the urine by physical, chemical or biochemical means to accelerate the aging process, and/or removing various urinary components by physical, chemical or biochemical means. In one embodiment, the body fluid is obtained from the pregnant female between the 5th and 15th weeks of pregnancy. Repeated analysis of urine samples obtained from the same test subject at different time points in the pregnancy appears to improve the accuracy of the analytical results. For example, if three urine samples are analyzed, we have found that the accuracy of the results approaches 100%.

The methods of the present invention also provide for a means for pre-conception baby gender planning by assaying the overall redox activity of a non-pregnant female's urine or other body fluid. The body fluid may be assayed at anytime during a menstrual cycle, but preferably is assayed around the time of ovulation or middle of the menstrual cycle. Without being limited to any specific physiological mechanism, it is believed that ovulation generates at least two types of physiological conditions during alternate menstrual cycles, presumably due to the release of two different types of ova. One type of ovum is compatible with fertilization by a Y chromosome-bearing sperm to produce a male fetus, whereas the other type of ovum is compatible with fertilization by an X chromosome-bearing sperm to produce a female fetus. The fertilization rate for a sperm and an incompatible ovum is very low. We have found that the overall redox activity of a urine sample correlates with the gender specific compatibility of the ovum being released during a particular menstrual cycle. Therefore, assaying the overall redox activity of a non-pregnant female's urine will help a couple conceive a baby having a desired gender.

The methods of the present invention may encompass assaying any suitable body fluid, including urine, tears, saliva, sweat, blood, plasma, serum, cerebrospinal fluid and amniotic fluid. Pigman et al. The reducing power of human saliva and its component secretions. *J. Dent Res.* 37(4): 688-696 (1958). Oyawoye et al. Antioxidants and reactive oxygen species in follicular fluid of women undergoing IVF. *Human Reproduction.* 18 (11): 2270-2274 (2003).

The term "overall redox activity" or "redox activity" of the body fluid refers to the net redox activity (including antioxidant activity) of a body fluid as a whole, although various components of the body fluid may exhibit different redox activities individually. The overall redox activity of the body fluid such as urine depends not only on the composition of the urine, but also, on how the sample is processed before assaying, as well as on the assay conditions. For example, the body fluid may be "aged" for at least about 1 week at room temperature ranging from about 20° C. to about 30° C. before assaying. The body fluid may also be aged for longer periods of time such as about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12 months. The body fluid may be aged at room temperature for a period of time ranging from about 1 week to about 52 weeks prior to assaying. Aging of the urine sample can range from as long as 1 year to about 5 years, but longer periods may also be appropriate. Other suitable chemical, biochemical or physical treatments may also be used to process the urine before assaying.

The term "fetal gender related characteristics" refers to (i) the evaluation of type and compatibility of ovum released in a particular menstrual cycle before pregnancy, and (ii) the determination of sex of the fetus after pregnancy. The term "gender specific compatibility" refers to the compatibility of the ovum with fertilization by either a Y chromosome-bearing

sperm to produce a male fetus, or by an X chromosome-bearing sperm to produce a female fetus.

The present invention provides a method for determining the gender of an unborn child comprising the steps of: (a) contacting a body fluid from a pregnant female with at least one redox indicator; (b) measuring redox activity of the body fluid; and, (c) comparing the redox activity of the body fluid with at least one standard to determine the gender of the unborn child.

Because the ability of the body fluid to reduce or oxidize a reagent will depend on the particular reagent (reducing agent or oxidizing agent) that is used to measure reduction or oxidation, the redox activity of the body fluid from a test subject is compared with the redox activity of a standard, i.e., the body fluid from a pregnant female where the sex of the fetus is known, to determine the gender of the fetus carried by the test subject. Alternatively, the redox activity of the body fluid from a test subject is compared with the redox activity of the pooled body fluid samples from pregnant females where the sexes of the fetuses are known, to determine the gender of the fetus carried by the test subject. Similarly, for pre-conception methods, the redox activity of the body fluid from a test subject is compared with the redox activity of a standard, i.e., body fluid from a non-pregnant female where the gender specific compatibility of the ovum the female generated is known, to determine the gender specific compatibility of the ovum produced by the test subject. Alternatively, the redox activity of the body fluid from a test subject is compared with the redox activity of the pooled body fluid samples from non-pregnant females where the gender specific compatibilities of the ova the females generated are known, to determine the gender specific compatibility of the ovum produced by the test subject. The redox activity of the standard may be determined using any method encompassed by this invention. One standard may be used in the present invention. Alternatively, more than one standard may be used.

The overall redox activity of the body fluid such as urine depends not only on the composition of the urine, but also, on how the sample is processed before assaying, as well as on the assay conditions. For example, if the urine sample is not aged or is assayed under mild conditions (see description below), the overall redox activity of the urine correlates with its in vivo overall redox activity. Namely, the urine from a female carrying a female fetus will have a comparatively higher overall reducing activity (or antioxidant activity) than urine from a female carrying a male fetus.

A similar observation has been made for pre-conception assays. Namely, if the urine sample is not aged or is assayed under mild conditions, the urine sample of a non-pregnant female carrying an ovum compatible with fertilization by an X chromosome-bearing sperm will have a comparatively higher reducing activity (or antioxidant activity) than urine from a non-pregnant female carrying an ovum compatible with fertilization by a Y chromosome-bearing sperm.

In contrast, if the urine sample is allowed to age and/or is assayed under harsh conditions (see description below), the urine would exhibit an overall redox activity different from its activity in vivo. Namely, the urine from a female carrying a male fetus will have a comparatively higher overall reducing activity than urine from a female carrying a female fetus.

Likewise, with pre-conception testing, if the urine sample is assayed under harsh conditions, the urine sample of a non-pregnant female carrying an ovum compatible with fertilization by a Y chromosome-bearing sperm will have a comparatively higher reducing activity than urine from a non-pregnant female carrying an ovum compatible with fertilization by an X chromosome-bearing sperm. See Table 1 for the redox activities of the urine samples under different processing and assay conditions.

TABLE 1

		Testosterone:Estrogens Ratio	Urine not aged or assayed under mild conditions	Urine aged or assayed under harsh conditions
Post-conception	Male Fetus	Greater than about 1	Comparatively higher overall oxidizing activity	Comparatively higher overall reducing (or antioxidant) activity
	Female Fetus	Less than about 1	Comparatively higher overall reducing (or antioxidant) activity	Comparatively higher overall oxidizing activity
Pre-conception	Ovum compatible with Y chromosome-bearing sperm	Greater than about 1	Comparatively higher overall oxidizing activity	Comparatively higher overall reducing (or antioxidant) activity
	Ovum compatible with X chromosome-bearing sperm	Less than about 1	Comparatively higher overall reducing (or antioxidant) activity	Comparatively higher overall oxidizing activity

The term "mild condition" refers to processing of the body fluid prior to assaying and assaying of the body fluid by treatment at temperatures lower than about 21° C. or treatment with mild chemicals, such as talc, alumina, extraction using organic solvents, solid phase extraction (SPE) chromatography or phosphotungstic acid (PTA). The term "harsh condition" refers to processing of the body fluid prior to assaying or assaying of the body fluid by treatment at temperatures greater than about 21° C. or treatment by harsh chemicals, such as strong acids, strong alkalis, trichloroacetic acid and strong oxidizing agents. Strong oxidizing agents include potassium ferricyanide in the ferric reducing/antioxidant power (FRAP) assay. The particular condition of the processing of the body fluid prior to assaying or assaying of the body fluid can be determined by one of ordinary skill in the art without undue experimentation.

There are a number of reducing or oxidizing compounds present in the urine that may interfere with the assays of the

present invention. Therefore, the urine may be processed to remove these compounds before assaying the redox activity. Interfering reducing and oxidizing agents include urea, creatine, uric acid, ascorbic acid, glucose, glucuronic acid, bilirubin, creatinine, porphyrins and related pigments, nitrites, sulfites, bisulfites, hyposulfites, pyrosulfites, sulfates oxyacids (such as sulfurous acid, bisulfurous acid, hyposulfurous acid, pyrosulfurous acid), urobilinogen, hemoglobin or other interfering proteins, and leucocytes. Interfering agents can be removed by treatment with enzymes including ascorbic acid oxidase, glucose oxidase, urease and uricase. Glucose may be removed by an anion exchange resin such as Amberlite-4B or alumina. Interfering agents may also include progesterone, its derivatives and/or metabolites, which may be removed by adsorbants such as mica and highly oriented pyrolytic graphite, or by antibodies against progesterone, its derivatives and/or metabolites. Ascorbic acid can be removed by resin extraction, diazotized 4-nitroaniline-2, 5-dimethoxyaniline, ascorbic acid oxidase, lead acetate, iodate, and/or their combinations. Michaelson. *Scand J Clin Lab Inves* 20: 97 (1967). Hughes. *Analyst.* 89:618 (1964).

Chemical, biochemical or physical treatments may also be used to remove the interfering reducing and/or oxidizing compounds before assaying. The treatments may include extraction, purification, adsorption, and treatments by mineral acids, enzymes or microwaving. The body fluid may also be processed by treatment with adsorbants and/or precipitants. Adsorbants may be selected from the group consisting of talc, silica-based particles such as silica gel, alumina, florisil, charcoal, kaolin, concanavaline A and its conjugates, calcium phosphate, calcium hydroxide, calcium chloride, Cetyltrimethyl ammonium bromide, lectin specific to the carbohydrate portion of urinary gonadotropins or glycoproteins, protein or glycoprotein hydrolyzing enzymes, glassfiber filter, ion exchange resins, affinity ligands, extraction using organic solvents, solid phase extractants, size exclusion sieves, and reverse phase chromatographic materials. Precipitants may be salts of heavy metals, which are selected from the group consisting of barium, lead, molybdenum and tungsten. Precipitants may comprise barium chloride, barium hydroxide mixed with zinc chloride, mercuric chloride, lead acetate and their mixtures. Precipitants may also comprise ammonium sulphate, dextran, acetonitrile, chloroform, sodium hydroxide, trichloroacetic acid, potassium iodate and their mixtures. One or more than one adsorbant or precipitant may be used; in addition, adsorbant and precipitant may be used in combination. Adsorbants or precipitants may be used to adsorb or retain the interfering reducing or oxidizing agents. Alternatively, adsorbants or precipitants may be used to adsorb or retain desired urinary fraction, which may later be eluted with appropriate solvents for assaying.

When the urine sample is allowed to age, most of the interfering reducing or oxidizing components appear to be eliminated. In one embodiment, the urine sample is first processed by physical, chemical or biochemical means to accelerate the aging process. The urine sample is then treated by other chemical, physical or biochemical means to obtain a purified or partially purified fraction, followed by assaying the overall redox activity of the urine sample under harsh conditions.

Prior to assaying, insoluble components may be removed from the body fluid, for example, by filtration or centrifugation. A non-interfering pH indicator may also be added prior to assaying. The pH of the body fluid may be adjusted.

The primary hormones directly linked to fetal gender are sex hormones, namely, testosterone and estrogens. The ratio of testosterone to estrogens is greater than about 1.0 in the urine from a mother carrying a male fetus, whereas the ratio of testosterone to estrogens is less than about 1.0 in the urine from a mother carrying a female fetus. Sex hormones, including testosterone and estrogen, are excreted in urine as their glucuronides associated with large glycoproteins such as human chorionic gonadotropin (hCG), sex hormone-binding globulin, as well as other glycoproteins. In freshly voided urine, sex hormones maintain their association with these large molecules. Without being limited to any specific physiological mechanism, it is believed that the overall redox activity of a urine sample assayed under mild conditions or without aging is the net redox activity of various urinary complexes which comprise sex hormones and large molecules such as hCG, sex hormone-binding globulin, as well as other glycoproteins.

When urine is assayed under harsh conditions, the hormones which can contribute to the reducing activity of a urine sample are progesterone, testosterone and human chorionic gonadotropin (hCG) and or its degradation byproducts, whereas estrogens can contribute to the oxidizing activity of the urine. hCG is a glycoprotein hormone specifically produced in pregnancy that is secreted by the embryo soon after conception and later by the placenta. hCG's major role is to maintain progesterone production that is critical for maintaining a pregnancy in humans. Many currently available early pregnancy tests are based on the detection or measurement of hCG in the blood or urine. The urine or blood level of hCG itself does not correlate with the gender of the fetus. hCG exhibits reducing activity. Nepomnaschy et al. *Human Reproduction.* 2008. 23(2): 271. Under harsh assay conditions, if a urine sample is from a female carrying a male fetus, there is no other stronger reducing agent (or antioxidant) in the urine sample that is able to protect hCG from being oxidized by urinary oxidizing agents. Bowman D. E. *J. Biol. Chem.* 1940, p 293-302. Gurin et al. *J. Biol. Chem.* 1940. 128: 525. As a result, hCG is oxidized and subsequently degraded to its constituents including galactoses and hexoamines, both of which have much stronger reducing activities than hCG. As a result, galactoses and hexoamines contribute to a comparatively higher overall reducing activity of the urine from a female carrying a male fetus in comparison with urine from a female carrying a female fetus. In contrast, in a freshly voided urine sample without aging or under mild assay conditions, if a urine sample is from a female carrying a female fetus, a relatively higher concentration of estrogen in the urine sample is able to protect hCG from oxidizing agents by interacting with the oxidizing agents. As a result, hCG is not oxidized or degraded to the same extent. Rather, hCG is degraded at a much slower rate in comparison to hCG in the urine sample from a female carrying a male fetus. Therefore, after aging or under harsh conditions, the urine from a female carrying a male fetus would exhibit a higher overall reducing activity than the urine from a female carrying a female fetus.

Sex hormones are excreted in urine as their glucuronide conjugates. As the urine sample is aged, glucuronide conjugates are hydrolyzed and free sex hormones released. To facilitate release of the free sex hormones from their glucuronide conjugates, a urine sample may be treated with. the enzyme β-glucuronidases. β-glucuronidases purified from various sources can be used, including bovine liver, snail *Helix pomatia* and *E. coli*. In one embodiment, the urine sample is treated with β-glucuronidases from *E. coli* for 24 hours. In another embodiment, the urine sample is treated with *E. coli* β-glucuronidases for 2 hours.

The urine sample may also be treated by a mineral acid to facilitate release of the free sex hormones from their glucuronide conjugates. The mineral acid may be hydrochloric acid

or sulfuric acid. The yield of hydrolysis is determined by the property and concentration of the acid. In one embodiment, a complete hydrolysis of the sex hormone glucuronide conjugates is achieved by treating a urine sample with 3 M sulfuric acid at 37° C. for 24 hours.

The redox potential of the body fluid may also be assayed. Redox potential may be determined by measuring the potential difference between an inert indicator electrode in contact with the body fluid and a stable reference electrode connected to the body fluid by a salt bridge. The inert indicator electrode acts as a platform for electron transfer to or from the reference half cell. The indicator electrode may comprise platinum. The indicator electrode may comprise gold and graphite. The reference electrode may be stable hydrogen electrode (SHE), Ag/AgCl reference electrode or saturated calomel (SCE) reference electrode.

Any suitable assay method, or a combination of two or more such assay methods, capable of measuring the overall redox activity of the body fluid may be used with the methods of the present invention. The overall redox activity of the body fluid may be assayed by reacting the body fluid with at least one redox indicator. As used herein, the term "redox indicator" refers to a molecule that undergoes a measurable change upon being reduced or oxidized. A redox indicator may be a reducing agent or oxidizing agent. The measurable change may be changes in color, fluorescence, chemiluminescence, electromagnetic radiation or any other suitable changes that may be assayed. The amount of the redox indicator reduced or oxidized may be directly correlated with the overall redox activity of the body fluid, which in turn is correlated with fetal gender, or the type of the ovum being produced. The redox indicator may be a chromogenic chemical capable of changing color upon being reduced or oxidized. The amount of the chromogenic chemical being reduced or oxidized can be measured by the difference in absorbances of the body fluid at a specific wavelength before and after the reduction/oxidation reaction.

Below are presented various types of redox assays that may be used to determine the overall redox activity of the body fluid such as urine. The overall redox activity of the body fluid may be determined by comparison with a standard where the sex of the fetus is known, or where the gender specific compatibility of the ovum is known. The comparative levels of the redox activity of the urine samples are shown in Table 1. The particular condition in each assay may be determined by a person of ordinary skill in the art without undue experimentation.

The overall reducing activity of a body fluid may be assayed using a heteropoly acid or its corresponding salts. Heteropoly acids are a class of acids each comprising a particular combination of metal, hydrogen, oxygen and other non-metal atoms. Specifically, a heteropoly acid contains a metal termed addenda atom, such as tungsten, molybdenum or vanadium; oxygen; a element termed hetero atom generally from the p-block of the periodic table, such as silicon, phosphorus or arsenic; and acidic hydrogen atoms. Two of the better known heteropoly acids are phosphotungstic acid (PTA) with the formula $H_3PW_{12}O_{40}$ and phosphomolybdic acid with the formula $H_3PMo_{12}O_{40}$.

The phosphotungstic and phosphomolybdic acids are very sensitive to reduction, and yield highly colored compounds even upon moderate reduction. Wu. Contributions to the chemistry of phosphomolybdic acid, phosphotungstic acid and allied substances. *J.* Biol. Chem. 1920. XLIII, 1: 189-220. Under acidic condition, PTA associates with proteins and forms precipitates in solution. Phosphotungstate: a "universal" (nonspecific) precipitant for polar polymers in acid solution. *Journal of Histochemistry and Cytochemistry*. J. E. Scott. 1971. 19, 11, 689. Besides proteins, PTA also has an affinity for carbohydrates at low pH. Pease DC. *J. Ultrastucture Res.* 1966. 15: 555. As pH of its solution increases, a heteropoly acid generally decomposes to its simpler constituent acids, but can be regenerated by re-acidification.

The assay using PTA may be conducted under mild conditions. With a PTA assay, the urine from a female carrying a female fetus will have a comparatively higher overall reducing activity as compared with urine from a female carrying a male fetus. When a urine sample from a pregnant female carrying a male fetus contacts PTA, PTA remains intact in the urine and forms insoluble complexes with urinary proteins and glycoproteins. Thus, this urine sample appears as a white colloidal suspension in the PTA assay for at least about 12 hours.

In contrast, for urine from a pregnant female carrying a female fetus, although PTA initially forms insoluble complexes with urinary proteins and glycoproteins, the complexes gradually decrease as PTA is being reduced by the urine. The reaction mixture becomes transparent over time. The time period for the reaction mixture to turn from a white colloidal suspension to clear solution may vary among different urine samples. In some embodiments, the time period may range from about 5 minutes to about 12 hours. Shorter or longer time periods may also be appropriate, such as about 1 minute, 2 minutes, 3 minutes, 4 minutes, 13 hours, 14 hours, 15 hours, 16 hours, 17 hours, 18 hours, 19 hours and 20 hours.

Tungsten or molybdenum is preferred as the addenda atom of the heteropoly acid or its salts used in the present invention. A heteropoly acid or its salts may consist of one or more than one types of addenda atoms. The combinations of the addenda atoms include molybdenum and tungsten, vanadium and molybdenum, and vanadium, molybdenum and tungsten. The hetero atom of a heteropoly acid or its salts is selected from the group consisting of phosphorus, arsenic, silicon and germanium. Phosphorus is preferred as the hetero atom. Heteropoly acids or their salts comprising molybdenum and phosphorus, molybdenum and arsenic, or tungsten and phosphorus are preferred embodiments. A heteropoly acid or its salts may be selected from the group consisting of phospho-12-molybdic acid, phospho-18-molybdic acid, 12-molybdoarsenic acid, 18-molybdoarsenic acid, 11-molybdo-1-vanadophosphoric acid, 10-molybdo-2-vanadophosphoric acid, phospho-12-tungstic acid, phospho-18-tungstic acid, phospho-24-tungstic acid and 9-molybdo-3-vanadophosphoric acid. Phospho-18-molybdic acid, phospho-12-tungstic acid, phospho-18-tungstic acid and phospho-24-tungstic acid are preferred embodiments. Heteropoly acids and their salts may be used individually or their combinations may be used.

The overall redox activity of a body fluid may be assayed by a chromogenic chemical comprising an oxidation-reduction sensitive metallic ion. The metallic ion may be selected from the group consisting of copper, iron, chromium, tungsten, molybdenum ions in an oxidized state.

The chromogenic chemical may be a metal-organic complex which, due to its extremely high oxidizing activity (correlated with high redox potential), can be reduced by many reducing agents and undergo change in color. The ferric reducing/antioxidant power (FRAP) assay can be used to assay the overall reducing activity of a body fluid. The ferric tripyridyltriazine (Fe(III)-TPTZ) complex is reduced under acidic conditions by a reducing agent to form ferrous tripyridyltriazine (Fe(II)-TPTZ). Fe(III)-TPTZ is yellow in color. The production of Fe(II)-TPTZ is easily detectable and mea-

surable because of its intense blue color with a maximum absorption at wavelength 593 nm.

The FRAP assay may be conducted under harsh conditions, where the urine from a female carrying a male fetus will have a comparatively higher overall reducing activity than urine from a female carrying a female fetus. Accordingly, a urine sample from a female carrying a male fetus is able to reduce Fe(III) to Fe(II), or Fe(III)-TPTZ to Fe(II)-TPTZ, with the reaction mixture turning dark blue. In contrast, a urine sample from a female carrying a female fetus is not able to reduce Fe(III), or Fe(III)-TPTZ, and thus, the reaction mixture remains yellow.

Potassium ferricyanide may also be used in the FRAP assay. Oyaizu. Studies on products of Browning reaction. *J. Nutrition.* 44: 307-315 (1986). Under the harsh conditions of the FRAP assay, the urine from a female carrying a male fetus will have comparatively higher overall reducing activity than urine from a female carrying a female fetus. Accordingly, a urine sample from a female carrying a male fetus is able to reduce potassium ferricyanide to potassium ferrocyanide, which is then able to form dark blue product when ferric chloride is added. In contrast, a urine sample from a female carrying a female fetus is not able to reduce potassium ferricyanide, and, thus, the reaction mixture remains golden yellow or faint green in color.

For fraternal multiples with different genders, when the FRAP assay is conducted, the urine with a comparatively higher overall reducing activity than urine from a female carrying a female fetus correlates with at least one male fetus, whereas the urine with comparable overall reducing activity to urine from a female carrying a female fetus correlates with at least two female fetuses.

The overall reducing activity of a body fluid may be assayed by the reduction of Cu^{2+} to Cu^{+}, which may be detected by the complex formation between Cu^{+} and bathocuproine. The amount of Cu^{+} being generated correlates with the overall reducing activity of the body fluid. The Cu^{+}-bathocuproine complex is stable and has a maximum absorption at wavelength around 480-490 nm. In one embodiment, the amount of Cu^{+} being generated is quantified by referring to a standard curve, which uses uric acid as the reducing agent.

The overall reducing or antioxidant activity of the body fluid may be assayed by its ability to capture a free radical in the presence of a redox indicator. In one embodiment, the radical is hydroxy radical (OH.) generated either by a hydroxyl radical-generating system or internally within the body fluid, with sodium salicylate as the redox indicator. In the absence of other antioxidant (or reducing agent), the colorless salicylate ion reacts with OH. to produce dihydroxybenzoic acid, which is blue in color and has a maximum absorption at wavelength 510 nm. When other antioxidant (or reducing agent) is present, hydroxyl radical is sequestered or trapped by this antioxidant (or reducing agent) instead. As a result, sodium salicylate stays intact and the reaction mixture remains colorless.

In another embodiment, the overall redox activity of the body fluid may be assayed by its ability to facilitate the generation of free radicals. The assay reagents may comprise sodium salicylate and a hydroxyl radical generating system including a ferric compound, such as ferric sulfate, and H_2O_2. When the reducing agent is present, it reduces ferric to ferrous, which results in further reduction of H_2O_2 to generate hydroxyl radical OH. through Fenton reaction. Salicylate ion then reacts with OH. to produce dihydroxybenzoic acid that is blue in color. The reaction is diagramed below.

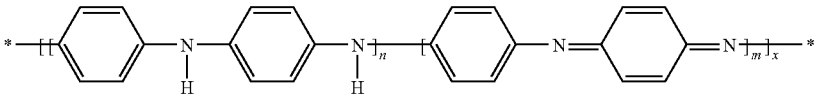
$$Fe^{3+}+\text{Reducing Agent}\rightarrow Fe^{2+}+\text{Oxidized Reducing Agent}$$

$$Fe^{2+}+H_2O_2\rightarrow Fe^{3+}+OH.+OH—$$

$$\text{Sodium Salicylate}+2OH.\rightarrow\text{Dihydroxybenzoic Acid}$$

In the absence of the reducing agent, no OH. is generated. As a consequence, sodium salicylate remains colorless.

The overall redox activity of the body fluid may also be detected using redox sensitive polymers such as polyaniline. Polyaniline is polymerized from the aniline monomer and has the following structure,



where x is half the degree of polymerization. Polyaniline can be found in one of three oxidation states. The leucoemeraldine form of polyaniline (n=1, m=0) is the fully reduced state and is white or colorless. Pernigraniline (n=0, m=1) is the fully oxidized state and is blue or violet. The emeraldine (n=m=0.5) form of polyaniline is the intermediate state and is green or blue. The color change associated with polyaniline in different oxidation states may be exploited to use polyaniline as a redox indicator. Huanga et al. Development and characterization of flexible electrochromic devices based on polyaniline and poly(3,4-ethylenedioxythiophene)-poly(styrene sulfonic acid). *Electrochimica Acta.* 51, 26: 5858-5863 (2006).

In another embodiment, the overall reducing activity of a body fluid may be assayed by an in situ methodology. For example, a redox indicator, which may be selected from the group consisting of thionine, toluidine blue O and cresyl violet, is immobilized to agarose beads. The agarose beads are then packed into a flow cell. The body fluid is allowed to pass through the flow cell and the redox states of the redox indicator is monitored by spectrophotometry. Jones. Evaluation of immobilized redox indicators as reversible, in situ redox sensors for determining Fe(III)-reducing conditions in environmental samples. *Talanta.* 2001. 55, 4: 699-714.

The overall redox activity of a body fluid may be detected by general reducing sugar tests using copper ion, neocuproine or alkaline ferricyanide method with or without color formation with o-phenanthroline complex. Prado et al. *Phytochem. Analysis.* 9 (2): 58-62 (1998).

The overall redox activity of the body fluid may also be detected by the Oxygen Radical Absorbance Capacity (ORAC) assay, which measures both lipophilic and hydrophilic antioxidant capacity using the same peroxyl radical generator. The ORAC assay measures the oxidative degradation of a fluorescent molecule (such as betaphycoerythrin or fluorescin) after being mixed with free radical generators such as azo-initiator compounds. Azo-initiator compounds produce peroxyl free radical, which damages the fluorescent molecule resulting in loss of fluorescence. Antioxidant or

reducing agent such as estrogen is able to protect the fluorescent molecule from the oxidative degeneration. The degree of protection by the antioxidant or reducing agent may be quantified using a fluorometer. Williams et al. A comparison of mammalian and plant estrogens on vascular reactivity in young and old mice with or without disruption of estrogen receptors. *Current Topics in Nutraceutical Res.* 2, 4: 191 (2004).

The overall redox activity of the body fluid may be detected by the Antioxidant Capacity (AOC) assay using the 3-ethylbenzothiazoline-6-sulfonate (ABTS) system or TPTZ. Bahramikia et al. A comparison of Antioxidant Capacities of ethanol extracts of S. Hortensis and A. Dracunculus leaves. *Pharmacology Online* 2: 694-704 (2008).

The overall redox activity of the body fluid may be detected by the use of immobilized chromogenic radicals. In one embodiment, either of two stable lipophylic chromogenic radicals, DPPH (2,2-diphenyl-1-picrylhydrazyl) radical and GV (2, 6-di-tert-butyl-α-(3,5-di-tert-butyl-oxo-2,5-cyclohexanedien-1-ylidene)-p-tolyloxy) radical, is immobilized in plasticized polymer films, such as polyvinyl chloride (PVC) films. When the polymer film containing immobilized 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical is reduced by a body fluid, it changes color irreversibly from purple (maximum absorption at wavelength 520 nm) to yellow. Steinberg et al. Chromogenic radical based optical sensor membrane for screening of antioxidant activity. *Talanta* 8: 15 (2006).

The overall redox activity of the body fluid may be detected by immunoassay such as immunofluorescence, using antibodies against gender related molecules; chemiluminescence; bioluminescence such as green fluorescent proteins; antibody-tagged gold nanoparticles of differing sizes; chemical tagging or fluorescent dyes. Kohen et al. Recent advances in chemiluminescence-based immunoassays for steroid hormones. *J. Steroid Biochem.* 27, 1-3: 71-79 (1987).

The overall oxidizing activity of the body fluid may be assayed by adding a source of ferrous ions to the body fluid, whereby oxidants in the sample oxidize at least a portion of the ferrous ions to ferric ions. Then a chromogenic compound is added to the sample, which reacts with at least a portion of the ferric ions. U.S. Patent Publication No. 20040259186.

The overall reducing activity of the body fluid may be assayed using reagent selected from the group consisting of ferricyanide salts, dichromate salts, permanganate salts, vanadium oxides, dichlorophenolindophenol, osmium bipyridine complexes, and quinones. The overall oxidizing activity of the body fluid may be assayed using reagent selected from the group consisting of iodine, triiodide salts, ferrocyanide salts, ferrocene, $[Cu(NH3)_4]^{2+}$ salts and $[Co(NH3)_6]^{3+}$ salts. The redox indicator of the present methods may be selected from the group consisting of 2,6-dichlorophenolindophenol (DCPIP), 3,3',5,5'tetramethylebenzidine (TMB), 1,4-phenylenediamine (DMPDA), Phenanthridine, 2,6-Dichloroindophenol, 2,2'-Azino-bis(3-ethylbenzthiazoline-6-sulfonic) acid, N,N-Dimethylphenylenediamine, 2-Amino-p-cresol (APC), Xylenol orange, 8-Hydroxy-7-iodo-5-quinoline-sulfonic acid and 4,5-Dihydroxy-1,3-benzene-di-sulfonic acid.

The present invention also provides a method for pre-conception baby gender planning by assaying the overall redox activity of a female's urine or other body fluid. The body fluid may be obtained anytime during a menstrual cycle, but preferably around the time of ovulation. Without being limited to any specific physiological mechanism, it is believed that there are two types of ova, each of which, in most of ovulating females, is generated in alternate months. One type of ovum is compatible with fertilization by a Y chromosome-bearing sperm to produce a male fetus, while the other type of ovum is compatible with fertilization by an X chromosome-bearing sperm to produce a female fetus. The overall redox activity of the maternal urine correlates with the gender specific compatibility of the ovum released in a particular menstrual cycle.

Under harsh assay conditions or with aging, the urine sample of a non-pregnant female carrying an ovum compatible with fertilization by a Y chromosome-bearing sperm will have comparatively higher reducing activity than urine from a non-pregnant female carrying an ovum compatible with fertilization by an X chromosome-bearing sperm (see Table 1 for summary). It is believed that a female produces two types of ova which generally alternate every month, i.e., if in one month the ovum being produced is compatible with fertilization by an X chromosome-bearing sperm, then in the next month the ovum being produced is compatible with fertilization by a Y chromosome-bearing sperm. The pre-conception urine assay can help a couple conceive a baby having a desired gender. For example, if a couple desires a baby girl, it is recommended that they plan to conceive in any month when the ovum being produced is compatible with fertilization by an X chromosome-bearing sperm. Similarly, if a couple wishes a baby boy, it is recommended that they plan to conceive in any month when the ovum being produced is compatible with fertilization by a Y chromosome-bearing sperm. The purpose of the present pre-conception methods is to raise the chances of conceiving the baby of desired gender by advising appropriate menstrual cycles for conception.

The present invention provides a method for pre-conception baby gender planning comprising the steps of: (a) contacting a body fluid from a non-pregnant female with at least one redox indicator; (b) measuring redox activity of the body fluid; and, (c) comparing the redox activity of the body fluid with at least one standard to determine gender-specific compatibility of the ovum released in a menstrual cycle. The body fluid may be obtained near ovulation or middle of the menstrual cycle. The body fluid may be processed prior to prior to step (a).

If two urine samples collected in two consecutive months manifest different redox activities, it suggests that the female has a regular alternating ovulation pattern as described above. If both urine samples demonstrate comparatively higher reducing activity than urine from a non-pregnant female carrying an ovum compatible with fertilization by an X chromosome-bearing sperm, the female may have an irregular ovulation pattern which is difficult to normalize according to the present invention. If both urine samples demonstrate comparatively higher oxidizing activity than urine from a non-pregnant female carrying an ovum compatible with fertilization by a Y chromosome-bearing sperm, the female's irregular ovulation pattern may be normalized through diet and exercise.

As described above, any suitable assay method, or a combination of two or more such assay methods, capable of measuring the overall redox activity of the body fluid may be used with the present pre-conception assay or the present post-conception assay.

The overall redox activity of the body fluid during a menstrual cycle within which a female conceives is similar to the redox activity of her body fluid after conception. Namely, if her pre-conception body fluid has a comparatively higher overall reducing activity than that of the body fluid from a non-pregnant female carrying an ovum compatible with fertilization by an X chromosome-bearing sperm under the harsh condition (for example, in the FRAP assay), her ovum is compatible with fertilization by a Y chromosome-bearing

sperm. Consistently, her post-conception urine sample would also have a higher overall reducing activity than that of urine from a female carrying a female fetus, which is correlated with a male fetus. Conversely, if her pre-conception urine sample has a higher overall oxidizing activity than that of urine from a non-pregnant female carrying an ovum compatible with fertilization by a Y chromosome-bearing sperm under harsh conditions, her ovum is compatible with fertilization by an X chromosome-bearing sperm. Likewise, her post-conception urine sample would also have a higher overall oxidizing activity than that of urine from a female carrying a male fetus, which is correlated with a female fetus.

The present invention further provides a method for determining the gender of a fetus that is within a female's uterus using remote detection devices. In vivo, a male fetus as a tissue mass has a higher overall oxidizing activity, or a lower reducing activity, than the surrounding maternal tissues, while a female fetus has a higher overall reducing or antioxidant activity than the surrounding maternal tissues. As is the case with post-conception or pre-conception urine tests, the redox activity of the fetal tissue or maternal tissue may be compared with a standard where the fetal gender is known. The remote detection device of the present invention may comprise a probe. The device may detect the redox activity of different parts of a mother's internal tissues by non-invasively sensing the internal electrochemical signals, electromagnetic signals, or any other suitable physical and/or chemical signals. In one embodiment, internal redox potentials are detected by the remote detection device. In another embodiment, internal electronic charges are detected by the remote detection device. In a further embodiment, the remote detection device comprises an electroscope to detect electrochemical potential of fetal and maternal tissues.

The present remote detection device may comprise broadband diffuse optical spectroscopy. Lee et al. Noninvasive in vivo monitoring of methemoglobin formation and reduction with broadband diffuse optical spectroscopy. *J. Appl. Physiology* 100: 615-622. As known in the art, oxidation of proteins and amino acids generates ultra weak photonemission. The present remote detection device may comprise a sensitive photomultiplier system to detect the ultra weak photon emission that is associated with a male or female fetus. The present remote detection device may comprise electron paramagnetic resonance imaging (EPRI) by using redox sensitive paramagnetic contrast agents. Yamada et el. Feasibility and assessment of non-invasive in vivo redox status using electron paramagnetic resonance imaging (EPRI). *Acta Radiol.* 43 (4): 433-40 (2002). The present remote detection device may comprise measuring the surface oxygen tension of organs. Miller A. T. Jr. University of North Carolina at Chapel Hill. Tissue oxygen tension and the intracellular oxidation-reduction state. Personal communication. Other suitable means may also be used for the present remote detection device. See, Wang. Remote electrochemical sensors for monitoring organic and inorganic pollutants. *Trends in Anal. Chem.* 16: 84 (1997). Balcom et al. Spatial and temporal visualization of two aqueous iron oxidation-reduction reactions by nuclear magnetic resonance imaging. *J. Chem Soc. Chem. Comm.* 1992: 312-313. Livesey et al. Measurement of tissue oxidation-reduction state with carbon-13 nuclear magnetic resonance spectroscopy. *Cancer Research.* 49: 1937-1940 (1989).

The change of the redox indicator may be analyzed with a scientific instrument, such as spectrophotometer, fluorometer, turbiditimeter, luminometer, fluorescence meter or colorimeter. The change in color of the chromogenic molecule may also be observed visually. The change of the redox indicator may be converted to digital signals which can be processed by digital, electronic or other sensing scientific instruments or materials. The scientific instrument may convert the assay results to a digital text response using any suitable means such as an electronic programming. In one embodiment, the instrument shows the text "It's a BOY" when the assay results suggest that the body fluid is from a female carrying a male fetus, and "It's a GIRL" when the assay results suggest that the body fluid is from a female carrying a female fetus. Similarly, for a pre-conception assay on the body fluid from a non-pregnant female, the instrument may show "Time for a baby boy" if the ovum produced in a particular menstrual cycle is compatible for fertilization by a Y chromosome-bearing sperm, and "Time for a baby girl" if the ovum produced in a particular menstrual cycle is compatible for fertilization by an X chromosome-bearing sperm.

The overall redox activity of the body fluid may be assayed using an electrochemical sensor, which is able to produce an electrochemically detectable signal upon being reduced or oxidized. In this case, a detection device may be used which comprises the redox indictor and has a disposable electrochemical cell, such as a thin layer electrochemical cell. The sensing electrode may consist of platinum, palladium, carbon, indium oxide, gold, iridium, copper, steel or mixture thereof. U.S. Pat. No. 6,638,415. WO2002/006806. Carbon nanotubes may be used for electrochemical sensing. Carbon nanotubes have the ability to promote electron transfer reactions when used as an electrode material in electrochemical reactions. U.S. Patent Publication No. 20060096870. Electrochemistry and electrogenerated chemiluminescence with a single faradaic electrode may be used for the present methods. It comprises of a faradaic working electrode and a capacitive counter electrode. When electrical energy is supplied in the presence of a body fluid, a faradaic charge transfer occurs wherein at least one of (i) light, (ii) current, (iii) voltage and (iv) charge can be measured to determine the presence or amount of analyte in a sample. WO2007/053191.

The overall redox activity of the body fluid may be assayed using any suitable chemical, physical, biochemical or biological means. The overall redox activity of the body fluid may be assayed by contacting the body fluid with at least one microorganism such as a bacterium, and observing the physiological responses such as generation of colored product, movement of the bacterium's cilia. The overall redox activity of the body fluid may be assayed by redox-sensitive green fluorescent protein (GFP). Dooley et al. Imaging dynamic redox changes in mammalian cells with GFP indicators. *J. Biol. Chem.* 279, 24: 22284-22293 (2004). The overall redox activity of the body fluid may be measured by assaying fluorescence. In one embodiment, 5-cyano 2-3 ditolyl tetrazolium chloride (CTC) is used for the present assay. CTC's oxidized form, which is colorless and non-fluorescent, is readily reduced to fluorescent, insoluble CTC-formazan form.

The present methods may be modified to allow many samples to be tested and results read simultaneously using, for example, a microplate or microarray.

The present invention also provides an article of manufacture such as a kit comprising (a) a urine collecting vial; (b) a solid substrate comprising a redox indicator, wherein the redox indicator comprises at least one chromogenic chemical; (c) printed material instructing a woman to collect urine and contact the urine with the solid substrate; and (d) printed material instructing a woman to compare redox activity of the urine sample with at least one standard to determine the gender of the unborn child based on change in color in the chromogenic chemical. The kit may also comprise other chemicals for processing and/or assaying the overall redox

activity of the body fluid. The printed matter may also indicate that, to determine the gender of an unborn child, the enclosed chemicals are to be used to assay the overall redox activity of the body fluid which will be compared with a standard. The printed material may instruct processing the urine prior to contact the urine with the solid substrate. The printed matter provides instructions as to how to use the enclosed chemicals for the test. Pictorial depictions of the instructions may be included in the printed matter. The printed matter may indicate that the body fluid is to be collected at any time during a pregnancy, starting from the first day of missed menstruation. The printed matter may also indicate that optimum results may be obtained if the body fluid is collected between the 5th and 15th week of pregnancy. The printed matter may include color standards for result interpretations and indicates the color (or a range of colors) that correlates with a male (or female) fetus. The chemicals may be provided in pre-measured quantities and may be in the form of solution, solid crystals or dry film. The chemicals may be placed in capped vials or bottles and placed in separate compartments. The chemicals may be absorbed or retained to solid supports and arranged sequentially to form a trickling column so as to ensure that various processes are conducted in a desired order. A kit may contain chemicals in sufficient amounts for one, two or more assays.

The present invention further provides an article of manufacture such as a kit comprising necessary chemicals for processing and/or assaying the overall redox activity of the body fluid such as urine, as well as printed matter indicating that, to determine the suitable menstrual cycle for conceiving a baby of desired gender, the enclosed chemicals are to be used to assay the overall redox activity of the body fluid in at least two consecutive menstrual cycles. The printed matter may indicate that the body fluid may be collected at any time during a menstrual cycle. The printed matter may also indicate that optimum results may be obtained if the body fluid is collected around the ovulation period. The printed matter provides instructions as to how to use the enclosed chemicals for the test. The printed matter also includes color standards for result interpretations and indicates the color (or a range of colors) that correlates with an ovum compatible with fertilization by a Y chromosome-bearing sperm or an ovum compatible with fertilization by an X chromosome-bearing sperm. The printed matter indicates that two types of ova are produced in alternate months, and suggests that a woman who wishes to have a baby boy (or girl) try to conceive in alternate months, starting from the month an ovum compatible with fertilization by a Y chromosome-bearing (or X chromosome-bearing) sperm is ovulated.

The redox activity may be measured on a solid substrate which comprises a redox indicator, such as a chromogenic chemical. The chromogenic chemical may be impregnated on a strip. The article of manufacture of the present invention may be a test strip. The test strip may have a sample application site where the body fluid may be applied. The body fluid may then pass through various aging, processing, filtering, adsorbing or chromatographic media, which ultimately leads to the procedure of assaying the overall reducing or oxidizing activity of the sample by reacting with at least one redox indicator. The test strip may have more than one sample application site so that the body fluid may be applied at more than one site on the strip at the same time. The same body fluid applied at different sites on the strip may undergo different treatments and may meet different chromogenic chemicals or redox indicators. The overall reducing or oxidizing activity of the sample is then evaluated based on a specific pattern of colors or other measurable signals.

The methods of the present methods may be used to test a body fluid obtained from any mammal, such as a human, horse, dog, monkey, sheep, pig and cat.

The Examples illustrate embodiments of the invention and are not to be regarded as limiting.

EXAMPLE 1

Post-Conception Assay

Table 2 tabulates the results for post-conception tests conducted for 4,524 women during the period between 1999 and 2008, with an average observed accuracy of about 90%.

At the initial stage, i.e., from the years 1999 through mid-2003, urine samples were tested using sodium salicylate as the redox indicator combined with spectrophotometry (see Example 4). PTA test (see Example 2) was started in 2002. FRAP assay (see Example 3) has been conducted since 2008.

TABLE 2

YEAR	Stage of pregnancy (wks)	Boys Observed	Boys Reported	Girls Observed	Girls Reported	Accuracy for Boys %	Accuracy for Girls %
1999-2001	7	212	242	229	258	87.6	88.8
	8	305	344	331	367	88.7	90.2
	9	299	338	332	378	88.5	87.8
	10 & up	281	328	293	345	85.4	84.9
2002	6	22	29	23	25	75.9	92.0
	7	21	31	18	19	67.7	94.7
	8	32	36	19	21	88.9	90.5
	9	17	21	20	22	81.0	90.9
	10 & up	46	54	26	31	85.2	83.9
2003	6	14	16	26	31	77.8	83.9
	7	26	33	16	18	78.8	88.9
	8	25	25	22	24	100.0	91.7
	9	16	16	13	13	100.0	100.0
	10 & up	37	45	20	21	82.2	95.2
2004	6	16	19	13	15	84.2	86.7
	7	26	35	19	22	74.3	86.4
	8	22	29	22	24	75.9	91.7
	9	9	12	12	14	75.0	85.7
	10 & up	16	21	26	31	76.2	83.9

TABLE 2-continued

YEAR	Stage of pregnancy (wks)	Boys Observed	Boys Reported	Girls Observed	Girls Reported	Accuracy for Boys %	Accuracy for Girls %
2005	5	10	11	5	5	90.9	100.0
	6	13	14	13	14	92.9	92.9
	7	21	26	20	24	80.8	83.3
	8	8	9	9	10	88.9	90.0
	9	9	10	12	13	90.0	92.3
	10 & up	48	58	48	51	82.8	94.1
2006	5	22	23	20	22	95.7	90.9
	6	30	33	24	25	90.9	96.0
	7	13	13	15	15	100.0	100.0
	8	15	15	11	13	100.0	84.6
	9	8	8	8	9	100.0	88.9
	10 & up	26	28	39	41	92.9	95.1
2007	5	19	21	32	36	90.5	88.9
	6	16	18	13	15	88.9	86.7
	7	16	18	17	18	88.9	94.4
	8	20	24	15	15	83.3	100.0
	9	12	13	12	14	92.3	85.7
	10 & up	39	42	45	48	92.9	93.8
2008	5	26	28	34	37	92.9	91.9
	6	25	25	39	42	100.0	92.9
	7	22	23	30	32	95.7	93.8
	8	23	25	21	22	92.0	95.5
	9	28	30	25	25	93.3	100.0
	10 & up	47	49	55	58	95.9	94.8
TOTAL		1,958	2,241	2,042	2,283	88%	91.3%
Total Fetal Gender Predicted			4,524			Average Observed Accuracy: 90%	

From 1999 to 2001 we tested urine samples collected after 21 days past missed menstruation date. Since 2005, we have been testing urine samples collected 1 day after missed menstruation, i.e., the 5th week of pregnancy.

If the urine samples were aged for a longer period of time, the accuracy of the present methods could approach 100%. However, from a clinical perspective, aging the urine samples for long period of time is not applicable. Accordingly, techniques to accelerate the aging process by physical, chemical or biochemical means were developed.

EXAMPLE 2

Processing of Urine Samples for the PTA Assay

For the PTA assay, the urine samples were collected and processed as follows. Post-conception urine samples were collected from about 2,782 females at various time points of a pregnancy ranging from 4 days before missed menstruation to the 20th week of pregnancy. The customers were shipped a vial for urine sample collection with a boric acid tablet in the vial as a preservative. Customers were asked to collect urine voided during a resting period including early morning for at least two days. Customers who were working at night were asked to collect urine during day-time instead for three consecutive resting periods. The samples were returned to the clinic by mail which took from 2 to 5 days depending upon the distance. The delivery of the urine sample by mail provided a time period for sample aging. The urine sample may be further aged at room temperature for longer periods of time.

The urine was then processed as follows. 2 ml of the urine sample was passed through acidic alumina to eliminate significant amount of pigments and glucose. A C 18 column was activated by methanol and pre-equilibrated with water. The cleared urine sample was then loaded on the C18 column to be subjected to solid phase extraction (SPE). After the urine sample passed through the C18 column, about 95% of sex hormones were retained on the column. The fraction of the urine sample that passed through or did not bind to the column was referred to as Fraction A. Fraction A was then treated with 50 ul of 10% Na_2HPO_4 and an excess amount of calcium phosphate (about 160 mg) to remove bilirubin, urobilinogen, porphyrins, and other pigments. 50 ul of saturated mercuric chloride was added to Fraction A to precipitate uric acid, ascorbic acid, creatine and some interfering proteins. 50 ul of 10% lead acetate solution was then added to Fraction A to remove creatine, some interfering proteins and bilirubin. 10 mg of barium chloride crystals was added to Fraction A to remove bilirubin, remaining interfering proteins and some interfering inorganic oxidants/reductants. The pH of Fraction A was then adjusted to 8.5 with 50 ul to 70 ul of 1 M sodium hydroxide, followed by centrifugation or filtration to remove the insoluble components.

In the PTA test, 20 mg of crystalline phosphotungstic acid was added to 0.75 ml of Fraction A, and the solution was kept below 21° C. for 12 hours. If the solution turned to a stable white fluorescent colloidal suspension after 12 hours, the urine sample was reported to be from a female carrying a male fetus. If the solution initially formed white precipitates which later disappeared to result in a transparent solution, the urine sample was reported to be from a female carrying a female fetus. Repeated analysis of the same urine sample aged for different periods of time improved the accuracy of the analytical results.

EXAMPLE 3

Processing of Urine Samples for a FRAP Assay

For the FRAP assay, the urine samples were processed as follows. The urine samples were treated as above in Example 2; however, in this case the components that were retained on the C18 column were eluted and tested.

2 ml of the urine sample was passed through acidic alumina to eliminate significant amount of pigments and glucose. A C18 column was activated by methanol and pre-equilibrated with water. The cleared urine sample was then loaded on the C18 column. After the urine sample passed through the C18 column, about 95% of sex hormones were retained on the column, which were than eluted with 1 ml of HPLC grade methanol and called Fraction B. Fraction B was diluted with 2 ml water. 2.5 ml of diluted Fraction B was mixed with 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixture was incubated at 50° C. for 20 min and cooled to room temperature. The mixture was then mixed with 2.5 ml of 10% trichloroacetic acid and centrifuged at 6500 rpm for 10 min. The supernatant (2.5 ml) was mixed with 2.5 ml distilled water, before 0.5 ml of 0. 1 % ferric chloride was added. In this assay, the results were read within one minute after the addition of the ferric chloride, as the results were not stable at later time points.

If Fraction B turned to deep blue after the FRAP assay, the urine sample was reported to be from a female carrying a male fetus. If Fraction B turned to golden yellow, faint yellow or faint green after the FRAP assay, the urine sample was reported to be from a female carrying a female fetus. Repeated analysis of the same urine sample aged for different periods of time improved the accuracy of the analytical results.

If the same urine sample was analyzed by more than one type of assay, we have found that the accuracy of the results can approach 100%. For example, if the PTA test on Fraction A produced a stable white fluorescent colloidal suspension after 12 hrs, and the FRAP test on Fraction B resulted in a deep blue solution, it was almost 100% accurate that the urine sample was associated with a male fetus. Similarly, if the PTA test on Fraction A produced a transparent solution after 12 hrs, and the FRAP test on Fraction B resulted in a solution with golden yellow, faint yellow or faint green in color, the accuracy was close to 100% that the urine sample was correlated with a female fetus.

Alternatively, the FRAP test can also be performed on Fraction A. When urine samples were aged for at least 3 months at room temperature, the accuracy of this method conducted was close to 100%.

EXAMPLE 4

Redox Activity Assay with Sodium Salicylate

The urine samples were processed and assayed using sodium salicylate as follows. For post-conception urine sample assays, customers were asked to collect early morning urine samples on three consecutive mornings and drop them off at the clinic. All three samples from the same customer were studied separately and a report was issued after pooling the results. To process the urine sample, 4 ml of urine sample was treated with 20 mg of lead acetate for 30 minutes to precipitate interfering proteins. 1 ml of processed urine sample and 1 ml of 0.75 M hydrochloric acid were added to a 10 ml glass vial, before 1 ml of 1% sodium salicylate was added slowly to the vial which was gently shaken to mix the reagents. Controls for the test were also included where, instead of 1 ml of 1% sodium salicylate, 1 ml of distilled water was added to the vial. The reaction mixture was incubated at 50° C. for 2 hours and then cooled to room temperature. The absorbance of the reaction mixture at wavelengths between 470 nm to 550 nm was then measured against control in a spectrophotometer. When a peak was observed around wavelength 510 nm, a result of a male fetus was recorded for this sample. When a trough or a straight base line was observed around wavelength 510 nm, a result of a female fetus was recorded for this sample.

EXAMPLE 5

FRAP Assay with Fe(III)-TPTZ

Fe(III)-TPTZ may also be used as the redox indicator for the FRAP assay. Under acidic conditions, Fe(III)-TPTZ complex can be reduced to Fe(II)-TPTZ. Fe(II)-TPTZ has intense blue color and can be monitored at wavelength 593 nm. FRAP working solution was freshly prepared by mixing 10 ml 300 mmol/1 acetate buffer (pH 3.6), 1.0 ml 10 mmol/1 TPTZ (2,4,6 tripyridyl-s-triazine) in 40 mmol/1 HCl solution, and 1.0 ml 20 mmol/1 $FeCl_3.6H_2O$ solution.

2 drops of processed urine sample were added to 0.5 ml FRAP working solution and the solution was incubated for 10 minutes. Under the harsh conditions of the FRAP assay, the urine from a female carrying a male fetus has a comparatively higher overall reducing activity than urine from a female carrying a female fetus. Accordingly, a urine sample from a female carrying a male fetus was able to reduce Fe(III)-TPTZ to Fe(II)-TPTZ, with the reaction mixture turned dark blue. In contrast, a urine sample from a female carrying a female fetus was not able to reduce Fe(III)-TPTZ, and thus, the reaction mixture remained yellow. When urine samples were aged for at least 3 months at room temperature, the accuracy of this method conducted on 161 urine samples was close to 100%. Among the 161 urine samples, 67 samples were from females carrying a male fetus; 94 samples were from females carrying a female fetus.

EXAMPLE 6

Redox Activity Assay with Polyaniline-Coated Film

Polyaniline-coated film was prepared by chemical deposition of polyaniline on a transparent film of poly(ethylene terephtahalate). 100 ml of the reaction mixture comprised 1 M sulfuric acid, 1 ml of aniline, 0.9 g of potassium iodate and 1 g of 5-sulfosalicyclic acid. U.S. Pat. No. 5,451,526. A deposition time of 2.5 hours at room temperature was used to produce the polyaniline film. The polyaniline coatings on both sides of the film were carefully washed to remove loose deposits. The film was dried, cut into small pieces (3 mm×2.0 cm), and stored in a clean container before use. Immediately prior to use, the film was equilibrated in an acidic ferric chloride solution for 2 minutes to convert all the polyaniline to its emaraldine state, which is the oxidized state. 1.75 ml of urine was processed by passing through 160 mg of acidic alumina column, and transferred to a cuvette. The Polyaniline-coated film was carefully washed with deionized water and transferred to the cuvette containing aged or processed urine. The optical density of the liquid in the cuvette was read at wavelength 630 nm on a spectrophotometer. Alternatively, after washing, the film was dipped in the purified urine fraction for 1 second before being taken out for visual observation of its change in color. Under the mild condition of this assay, the aged and processed urine from a female carrying a female fetus has a comparatively higher overall reducing activity than urine from a female carrying a male fetus, and therefore, was able to reduce the emeraldine form of polyaniline (blue) to its more reduced leucoemeraldine state (off-white). An aged and processed urine sample from a female bearing a male fetus did not react with the emeraldine form of polyaniline (blue). As a result, the film remained blue in color.

When fresh urine samples were used without processing, no difference could be observed in this test.

EXAMPLE 7

Pre-conception Assay

The overall redox activity of the body fluid during a menstrual cycle within which a female conceives is similar to the redox activity of her body fluid after conception. Namely, if her pre-conception body fluid has a comparatively higher overall reducing activity than that of the body fluid from a non-pregnant female carrying an ovum compatible with fertilization by an X chromosome-bearing sperm under the harsh condition (for example, in the FRAP assay), her ovum is compatible with fertilization by a Y chromosome-bearing sperm. Consistently, her post-conception urine sample would also have a higher overall reducing activity than that of urine from a female carrying a female fetus, which is correlated with a male fetus. Conversely, if her pre-conception urine sample has a higher overall oxidizing activity than that of urine from a non-pregnant female carrying an ovum compatible with fertilization by a Y chromosome-bearing sperm under harsh conditions, her ovum is compatible with fertilization by an X chromosome-bearing sperm. Likewise, her post-conception urine sample would also have a higher overall oxidizing activity than that of urine from a female carrying a male fetus, which is correlated with a female fetus.

The FRAP assay was used to test both pre-conception and post-conception urine samples obtained from the same customer. Pre-conception urine samples were collected during ovulation period for at least two consecutive months. Post-conception urine samples were collected on the first or second day of missed menstruation. If the pre-conception samples indicated an alternating ovulation pattern, then the customer were advised as follows. If the customer desired a baby boy, she was advised to try conception in months when the ova being produced were compatible with fertilization by a Y chromosome-bearing sperm. If the customer desired a baby girl, she was advised to try conception in months when the ova being produced were compatible with fertilization by an X chromosome-bearing sperm.

Those customers whose urine samples indicated ova being released in two consecutive months had the same gender specific compatibilities were advised to collect more urine samples for additional tests. However, customers of this category did not wish to incur more costs and, therefore, did not continue after the initial tests.

Table 3 tabulates the results of pre-conception tests we conducted for 130 customers during the period between 2006 and August 2008.

TABLE 3

Number of customers who received the pre-conception test and later the post-conception test = 130			
Number of customers who desired a baby boy and tried conception according to our method = 71	Number of customers who confirmed having a baby boy = 52 (Few did not call back*.)	Number of customers who desired a baby girl and tried conception according to our method = 59	Number of customers who confirmed having a baby girl = 41 (Few did not call back*.)
Success rate for conception of baby boy = 73.2%		Success rate for conception of baby girl = 69.5%	
Total success rate of pre-conception test = 71.3%			

*As a policy, we never call a customer for a feedback due to confidentiality concerns.

Examples 8-12 illustrate various methods to process urine samples before assaying its overall redox activity.

EXAMPLE 8

Processing of Urine Samples with Acidic Alumina, Calcium Chloride and Lead Acetate

The pH of 3 ml urine sample was adjusted to 6.4 with 1 M HCl. The urine sample was then centrifuged to remove insoluble components. The supernatant was loaded to a column containing 200 mg acidic alumina to remove interfering agents. 50 ul of 10% Na_2HPO_4 and 80 mg of calcium chloride were then added to the cleared urine sample. The reaction mixture was incubated at room temperature for 5 minutes. 10 mg of lead acetate was added to the sample and the test tube was shaken until all lead acetate dissolved. After 5 minutes, the pH of the sample was adjusted to 8.5 with 2.5 M NaOH. The sample was then centrifuged at 12000 rpm for 2 minutes to remove insoluble components. The processed urine sample was then assayed using the PTA or FRAP assay (Examples 2 and 3).

EXAMPLE 9

Processing of Urine Samples with Sulfuric Acid and Florisil

1 ml of fresh urine was added to a microcentrifuge tube and incubated on ice. Concentrated sulfuric acid was slowly added to the urine sample to reach its final concentration of 3 M in the urine sample. The microcentrifuge tube was inverted slowly several times and the mixture incubated at 37° C. for 24 hours. The sample was then centrifuged at 12000 rpm for 2 minutes before the supernatant was passed through Florisil to result in processed urine sample. 10 mg of crystalline phospho-24-tungstic acid was then added to 0.75 ml of this processed urine sample, and the PTA assay conducted (Example 2).

EXAMPLE 10

Processing of Urine Samples with Ion Exchange Resins

The pH of 2 ml of urine sample was adjusted to pH 5.3 with diluted glacial acetic acid. The urine sample was then centrifuged at 12000 rpm for 2 minutes. The supernatant was batch treated with 160 mg of well washed Dowex™ resins (Dow Chemical Company) 1X 8-100 mesh Cl^- form ion exchange resin to remove porphyrins. This cleared urine sample was

then processed as described in Example 8. The processed urine sample was assayed using the PTA or FRAP assay (Examples 2 and 3).

EXAMPLE 11

Processing of Urine Samples with Nylon Filter

The pH of 2 ml of urine sample was adjusted to 4.0 with 1 M HCl, before the urine sample was centrifuged at 12000 rpm for 2 minutes. The supernatant was passed through 0.2 um Nylon filter to remove porphyrins. This cleared urine sample was then processed as described in Example 8.

EXAMPLE 12

Processing of Urine Samples with Acidic Alumina and Talc

In order to develop a home based gender test, 2 ml of urine sample was added to 200 mg of acidic alumina and talc, and the tube was shaken to ensure mixing. The sample was incubated at room temperature for 5 minutes, before the sample was filtered to obtain processed urine sample. The processed urine sample was assayed using the FRAP assay (Example 3).

The scope of the present invention is not limited by what has been specifically shown and described hereinabove. Those skilled in the art will recognize that there are suitable alternatives to the depicted examples of materials, configurations, constructions and dimensions. Numerous references, including patents and various publications, are cited and discussed in the description of this invention. The citation and discussion of such references is provided merely to clarify the description of the present invention and is not an admission that any reference is prior art to the invention described herein. All references cited and discussed in this specification are incorporated herein by reference in their entirety. Variations, modifications and other implementations of what is described herein will occur to those of ordinary skill in the art without departing from the spirit and scope of the invention. While certain embodiments of the present invention have been shown and described, it will be obvious to those skilled in the art that changes and modifications may be made without departing from the spirit and scope of the invention. The matter set forth in the foregoing description and accompanying drawings is offered by way of illustration only and not as a limitation.

What is claimed is:

1. A method of determining the gender of an unborn child comprising the steps of:

(a) obtaining a urine sample from a pregnant female during a period ranging from about 4 days before missed menstruation to about 40 weeks of pregnancy;

(b) aging the urine sample at room temperature for at least about 2 days;

(c) processing the urine sample by a method selected from the group consisting of: (i) treating the urine sample with a precipitant comprising a heavy metal element selected from the group consisting of barium, lead, molybdenum, tungsten, zinc and mercury; (ii) treating the urine sample with an adsorbant selected from the group consisting of calcium phosphate, alumina, talc, kaolin, florisil, calcium chloride, nylon, ion exchange resins, silica gel, charcoal, concanavaline A and its conjugates, calcium hydroxide, Cetyltrimethyl ammonium bromide, lectin, a protein or glycoprotein hydrolyzing enzyme, an affinity ligand, a solid phase extractant, size exclusion sieves, and reverse phase chromatographic materials; (iii) treating the urine sample with a β-glucuronidase; and (iv) a combination of any of (i), (ii), and (iii);

(d) contacting the processed urine sample with at least one redox indicator; and

(e) detecting redox activity of the processed urine sample by a chromogenic assay selected from a PTA assay or FRAP assay.

2. The method of claim **1**, wherein the urine sample is obtained during a period ranging from about 5 weeks to about 15 weeks of pregnancy.

3. The method of claim **1**, wherein the redox indicator comprises a heteropoly acid or its salts.

4. The method of claim **3**, wherein the heteropoly acid is phospho-24-tungstic acid.

5. The method of claim **1**, wherein the redox indicator is a chromogenic chemical.

6. The method of claim **5**, wherein the chromogenic chemical comprises an oxidation-reduction sensitive metallic ion.

7. The method of claim **6**, wherein the metallic ion is selected from the group consisting of copper, iron, chromium, tungsten and molybdenum ions in an oxidized state.

8. The method of claim **5**, wherein the chromogenic chemical is selected from the group consisting of ferric tripyridyltriazine (Fe(III)-TPTZ) complex and potassium ferricyanide.

9. The method of claim **1**, wherein the redox activity is measured using a free radical.

10. The method of claim **9**, wherein the free radical is a hydroxyl radical.

11. The method of claim **9**, wherein sodium salicylate is used as the redox indicator.

12. The method of claim **1**, wherein the redox activity is measured using an electrochemical sensor.

13. The method of claim **1**, wherein the redox activity is measured on a solid substrate which comprises a redox indicator.

14. The method of claim **13**, wherein the redox indicator is a chromogenic chemical.

15. The method of claim **14**, wherein the chromogenic chemical is impregnated on a strip.

16. The method of claim **1**, wherein the aging is performed for about 1 week to about 4 weeks at room temperature ranging from about 20° C. to about 30 ° C. in step (b).

17. The method of claim **1**, wherein the aging is performed at room temperature for a period of time ranging from about 1 week to about 2 months in step (b).

18. The method of claim **1**, wherein the precipitants are selected from the group consisting of barium chloride, barium hydroxide, zinc chloride, mercuric chloride, lead acetate, and their mixtures.

* * * * *